Refine Search

Search Results -

Terms	Documents
L35 with L24	35

Database: US Pre-Grant Publication Full-Text Database; US Patents Full-Text Database; US OCR Full-Text Database; EPO Abstracts Database; JPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L36

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Search History

DATE: Wednesday, February 16, 2005 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT;PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L36	L35 with L24	35	L36
L35	L34 with L19	402	L35
L34	isolated	566953	L34
L33	L32 same L21	40	L33
L32	L30 with L19	958	L32
L31	L30 with L20 with L24	83	L31
L30	fibroblast	50132	L30
L29	5266480.pn.	2	L29
L28	L27 same L23	4	L28
L27	gene therapy	48237	L27
L26	L24 same L23	19	L26
L25	L24 with L23	7	L25
L24	vector or gene construct or plasmid or retrovir$	362805	L24

L23	L22 with L19	533	L23
L22	L21 or L20	3785863	L22
L21	implanta$	168958	L21
L20	polymer or chamber or microparticle or micrsphere or matrix or microcarrier	3685162	L20
L19	stromal cell	5397	L19
L18	cell	1303230	L18
L17	stromal	8902	L17
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L16	L15 and l14	138	L16
L15	cytotoxic or ganciclovir or thymidine	71277	L15
L14	L13 and l12	145	L14
L13	implant$ or polymer or chamber or matrix or container or diffu$	5643087	L13
L12	L11 and l8	161	L12
L11	promoter or gene therapy	179762	L11
L10	l9 and l8	237	L10
L9	stromal	8908	L9
L8	L7 same l6	237	L8
L7	hsv-tk or cytotoxic or cytosine deaminase or thymidine kinase or genotoxic or toxin	86812	L7
L6	L5 or l2	5608	L6
L5	L4 with l3	463	L5
L4	adherent	77317	L4
L3	bone marrow	35409	L3
L2	stromal cell	5398	L2
L1	5716616	20	L1

END OF SEARCH HISTORY

Refine Search

Search Results -

Terms	Documents
L15 and L14	138

Database: US Pre-Grant Publication Full-Text Database; US Patents Full-Text Database; US OCR Full-Text Database; EPO Abstracts Database; JPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Search: L16

Refine Search | Recall Text | Clear | Interrupt

Search History

DATE: Wednesday, February 16, 2005 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ			
L16	L15 and l14	138	L16
L15	cytotoxic or ganciclovir or thymidine	71277	L15
L14	L13 and l12	145	L14
L13	implant$ or polymer or chamber or matrix or container or diffu$	5643087	L13
L12	L11 and l8	161	L12
L11	promoter or gene therapy	179762	L11
L10	l9 and l8	237	L10
L9	stromal	8908	L9
L8	L7 same l6	237	L8
L7	hsv-tk or cytotoxic or cytosine deaminase or thymidine kinase or genotoxic or toxin	86812	L7
L6	L5 or l2	5608	L6
L5	L4 with l3	463	L5

L4	adherent	77317	L4
L3	bone marrow	35409	L3
L2	stromal cell	5398	L2
L1	5716616	20	L1

END OF SEARCH HISTORY

L10: Entry 52 of 237 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030165475
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030165475 A1

TITLE: Non-lethal methods for conditioning a recipient for bone marrow transplantation

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ildstad, Suzanne T.	Wynewood	PA	US	

US-CL-CURRENT: 424/93.7; 424/144.1, 514/110

CLAIMS:

What is claimed is:

1. A method for conditioning a recipient for bone marrow transplantation comprising subjecting the recipient to treatment with a dose of total body irradiation from 50 cGy to 550 cGy, followed by transplantation with a donor cell preparation containing hematopoietic stem cells which are not compatible with the recipient at the major histocompatibility complex, to achieve stable engraftment of donor hematopoietic stem cells, without the development of lethal graft-versus-host disease.

2. The method of claim 1 in which the recipient is further treated with an alkylating agent before, during, or after total body irradiation.

3. The method of claim 2 in which the alkylating agent is cyclophosphamide.

4. The method of claim 3 in which the cyclophosphamide is administered at a dose of between 50 mg/kg and 250 mg/kg.

5. The method of claim 1 in which the recipient is further treated with an antibody or an active fragment thereof before, during, or after total body irradiation.

6. The method of claim 5 in which the antibody is reactive with the CD8 cell surface marker.

7. The method of claim 5 in which the antibody is reactive with the CD4 cell surface marker.

8. The method of claim 5 in which the recipient is further treated with an alkylating agent.

9. The method of claim 1 in which the donor cell preparation is obtained from a human.

10. The method of claim 1 in which the donor cell preparation is obtained from a non-human primate.

11. The method of claim 1 in which the donor cell preparation is obtained from a pig.

12. The method of claim 1 in which the donor cell preparation further comprises hematopoietic facilitatory cells having a phenotype of CD8.sup.+, .alpha..beta.TCR.sup.-, and .delta..gamma.TCR.sup.-.

13. The method of claim 1 in which the donor cell preparation has been depleted of graft-versus-host-disease producing cells.

14. A method for conditioning a recipient for bone marrow transplantation comprising treating the recipient with antibodies or active fragments thereof directed to the CD8 or CD4 cell surface markers, singly or in combination, followed by transplantation with a donor cell preparation containing hematopoietic stem cells which are not compatible with the recipient at the major histocompatibility complex, to achieve stable engraftment of donor hematopoietic stem cells, without the development of lethal graft-versus-host disease.

15. The method of claim 14 in which the recipient is further treated with an alkylating agent before, during, or after total body irradiation.

16. The method of claim 15 in which the alkylating agent is cyclophosphamide.

17. The method of claim 16 in which the is administered at a dose of between 50 mg/kg and 250 cyclophosphamide mg/kg.

18. The method of claim 14 in which the recipient is further treated with a dose of total body irradiation from 50 cGy to 550 cGy.

19. The method of claim 14 in which the donor cell preparation is obtained from a human.

20. The method of claim 14 in which the donor cell preparation is obtained from a non-human primate.

21. The method of claim 14 in which the donor cell preparation is obtained from a pig.

22. The method of claim 14 in which the donor cell preparation further comprises hematopoietic facilitatory cells having a phenotype of CD8.sup.+, .alpha..beta.TCR.sup.-, and .delta..gamma.TCR.sup.-.

23. The method of claim 14 in which the donor cell preparation has been depleted of graft-versus-host-disease producing cells.

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L10: Entry 64 of 237 File: PGPB Jun 19, 2003

DOCUMENT-IDENTIFIER: US 20030114379 A1
TITLE: Methods of treating or preventing cell, tissue, and organ damage using human myeloid progenitor inhibitory factor-1 (MPIF-1)

Detail Description Paragraph:
[0490] Damage may be caused by depletion of potentially mitotic cells (known as the stem cell model); vascular injury causing hypoxia and other effects; normal host repair responses including induction of immediate early genes such as Jun and EGR1, induction of proinflammatory cytokines such as interleukins and TNF, induction of inflammatory cytokines such as TGF.beta., PDGF, BFGF, and induction of secondary cytokine cascade(s); effects of inflammatory responses; interactions between multiple cell types such as inflammatory cells, stromal functional cells and fibroblasts

Detail Description Paragraph:
[0492] Fibrosis may be induced in one or more ways: monocytes and macrophages present in the irradiated tissue are induced to produce proinflammatory cytokines, thus recruiting additional macrophages in an inflammatory response; the initial loss of epithelial and stromal cells induces inflammation; irradiation induces expression of fibrogenic cytokines through induction of AP-1.

Detail Description Paragraph:
[0531] Additionally, mesenchymal cells, stromal cells, hair cells/follicles, adipose (fat) cells, cells of simple epithelial tissues (squamous epithelium, cuboidal epithelium, columnar epithelium, ciliated columnar epithelium and pseudostratified ciliated columnar epithelium), cells of stratified epithelial tissues (stratified squamous epithelium (keratinized and non-keratinized), stratified cuboidal epithelium and transitional epithelium), goblet cells, endothelial cells of the mesentery, endothelial cells of the small intestine, endothelial cells of the large intestine, endothelial cells of the vasculature capillaries, endothelial cells of the microvasculature, endothelial cells of the arteries, endothelial cells of the arterioles, endothelial cells of the veins, endothelial cells of the venules, etc., and endothelial cells of the bladder may be treated with MPIF-1 to reduce or prevent cytotoxic damage.

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L10: Entry 74 of 237 File: PGPB Mar 27, 2003

PGPUB-DOCUMENT-NUMBER: 20030059412
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030059412 A1

TITLE: ISOLATED STROMAL CELLS AND METHODS OF USING THE SAME

PUBLICATION-DATE: March 27, 2003

US-CL-CURRENT: 424/93.21; 435/352, 435/372, 435/440, 435/455, 536/23.5, 536/24.1, 536/24.2

APPL-NO: 08/ 913918 [PALM]
DATE FILED: December 8, 1997
CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

PCT-DATA:

DATE-FILED	APPL-NO	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
Mar 28, 1996	PCT/US96/04407				

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L10: Entry 113 of 237 File: PGPB Mar 14, 2002

DOCUMENT-IDENTIFIER: US 20020031824 A1
TITLE: Method for homing hematopoietic stem cells to bone marrow stromal cells

Abstract Paragraph:
This invention pertains to a method for homing hematopoietic stem cells to bone marrow stromal cells in a host. The method comprises, administering to the host genetically-engineered hematopoietic stem cells capable of expressing a first member of a ligand-receptor binding pair. The stem cells are administered to the host under conditions whereby binding of the first member of the ligand-receptor binding pair to the second member of the ligand-receptor binding pair, present on stromal cells, occurs thereby homing the stem cells to the stromal cells. This method is useful for transplanting bone marrow in a host or in treating a host afflicted with a disease associated with a disorder of the bone marrow.

Summary of Invention Paragraph:
[0002] Long-term bone marrow cultures (LTBMC) contain two major cell populations referred to as compartments. The hematopoietic stem cell compartment, contains cells at various stages of self renewal capacity and differentiation and the adherent cell or stromal cell compartment has been shown to provide the environment necessary for the production and differentiation of hematopoietic stem cells and their progenitors. Both compartments interact to facilitate hematopoiesis in vitro (Naparstek, et al; Exp. Hematol. 13: 701-708 (1985).

Summary of Invention Paragraph:
[0003] Bone marrow transplants are widely used for treating congenital disorders of the bone marrow or hematopoietic stem cell, e.g. aplastic anemia, acute leukemias, recurrent lymphomas, or solid tumors. Prior to receiving a bone marrow transplant, the recipient is prepared by ablating or removing endogenous recipient hematopoietic stem cells. This preparation is usually carried out by total body irradiation or delivery of a high dose of an alkylating agent or other chemotherapeutic cytotoxic agents (Greenberger, J. S., Br. J. Hematol, 62: 606-605, 1986; Anklesaria, P., et al, PNAS, USA, 84: 7681-7685, 1987; Thomas, E. D., Cancer, 49: 1963, 1982; Thomas, E. D., N. Eng. J. Med., 292: 832-843, 895-902, 1975). Following preparation of the recipient, donor bone marrow cells are injected intravenously and have been demonstrated to home to multiple sites within the recipient where they proliferate and reconstitute all elements of the hematopoietic stem cell compartment including neutrophilic granulocytes, megakaryocytes (platelets), red blood cell progenitors leading to mature erythrocytes, T-lymphocytes, B-lymphocytes, monocyte/macrophages, basophils and mast cells (Thomas, E. D., cited supra). Some data from several clinical transplanation centers suggest that donor origin stromal cells of the hematopoietic microenvironment are also detected in small numbers in recipients after marrow transplant (Thomas, E. D., cited supra).

Summary of Invention Paragraph:
[0008] This invention pertains to a method for homing hematopoietic stem cells to bone marrow stromal cells in a host comprising, administering to the host genetically-engineered hematopoietic stem cells capable of expressing a first member of a ligand-receptor binding pair. The stem cells are administered to the host under conditions whereby binding of the first member of the ligand-receptor

binding pair to the second member of the ligand-receptor binding pair, present on stromal cells, occurs thereby homing the stem cells to the stromal cells.

Summary of Invention Paragraph:
[0009] Another embodiment of the method for homing hematopoietic stem cells to bone marrow stromal cells in a host can comprise a first step of administering to the host stromal cells capable of expressing a first member of a ligand-receptor binding pair followed by a second step of administering hematopoietic stem cells capable of expressing a second member of a ligand-receptor binding pair. In this embodiment, either the stem cells or stromal cells are genetically-engineered to provide the capability of expressing the appropriate ligand or receptor. The methods of this invention can be used for transplanting bone marrow in a host or for treating a host afflicted with a disease associated with a disorder of the bone marrow.

Summary of Invention Paragraph:
[0010] Transplantation of a donor bone marrow microenvironment, including both the stromal and stem cells, can reduce the risk of graft failure in instances where a defect in a patient's stroma is causing the graft failure. A problem encountered when attempting to transplant two cell lines which are dependent on each other is that there are multiple possible sites in a host where the cells can, "home". Thus, there is a chance that the cell lines may not "home" together. The method of this invention provides a method for homing stem cells to the engrafted microenvironment thereby allowing proliferation of the stem cells at these new sites to produce all the formed elements of the blood. The method of homing stem cells to stromal cells is advantageous in that it can be used to reconstitute the marrow of patients who have damaged marrow stroma and stem cells due to injury, congenital defects, or cytotoxic therapy.

Brief Description of Drawings Paragraph:
[0011] FIG. 1 is a schematic showing the transfection of a stromal cell line with an expression vector containing the Mo-MuLV LTR, the entire coding region (595 bp) for the mature 50 amino-acid transforming growth factor .alpha. (TGF.alpha.), and the neomycin gene which confers G418 resistance to mammalian cells. The transfection of a hematopoietic cell line with an expression vector containing cDNA for epidermal growth factor receptor (EGF-R), the Mo-MuLV LTR, and the E. coli GPT gene which confers resistance to mycophanolic acid is also depicted in the schematic.

Brief Description of Drawings Paragraph:
[0012] FIG. 2 is a photograph of a Northern blot analysis of total mRNA from the GPTGF.alpha. (GPT.alpha.), GBL/6 and EuT cell lines, for detectable transcripts for TGF.alpha.. EuT cells were used as the positive controls. Total mRNA (10 .mu.gs/lane) was run on a 1% agarose gel in 1% formaldehyde and transferred to a nylon membrane. Filters were hybridized with a .sup.32P labelled specific cDNA (542 bp insert), washed at high stringency and autoradiograms exposed for three to four days at -70.degree. C. with intensifying screens. A 4.8 Kb specific message was detected in the GPTGF.alpha. cells compared to the GBL/6 cells. This data, in addition to the biochemical evidence for release of TGF.alpha. in the conditioned medium of GPTGF.alpha. cells, provides strong evidence for the expression of TGF.alpha. in the GBL/6 stromal cell line.

Brief Description of Drawings Paragraph:
[0013] FIGS. 3A and 3B are graphs depicting the support of 32D-EGFR cells by a TGF.alpha. producing GPTGF.alpha. stromal cell line. The 32D-EGF-R cells (2.5.times.10.sup.6) were cocultivated with confluent cultures of GBL/6 or GPTGF.alpha. cells. At weekly intervals, "cobblestone areas" were scored and non-adherent cells were harvested and counted. The flasks were refed by adding an equal volume of fresh medium. The results are expressed as the mean .+-.SD of three flasks per experiment. FIG. 3A shows the cumulative number of "cobblestone areas"

per flask for over fifty days in culture. FIG. 3B shows the cumulative production of non-adherent cells/flask for over fifty days in culture. Factor-dependent 32D-EGF-R cells plated without interleukin-3 (IL-3) or epidermal growth factor (EGF) or stromal cells were not viable at four days in culture and did not adhere to plastic. There was a strong and clear effect that GPTGF.alpha. cells support 32D-EGFR cells in vitro in the adherent population and growth after detachment.

Detail Description Paragraph:
[0019] This invention pertains to a method for homing hematopoietic stem cells to bone marrow stromal cells in a host. The method comprises administering to the host genetically engineered hematopoietic stem cells capable of expressing the first member of a ligand receptor binding pair under conditions whereby binding of the first member of the ligand receptor binding pair to a second member of the ligand receptor binding pair, present on stromal cells, occurs thereby homing the stem cells to the stromal cells.

Detail Description Paragraph:
[0020] When it is desired to replace the stroma in a host, the method comprises a step of administering to the host stromal cells capable of expressing a first member of a ligand receptor binding pair followed by hematopoietic stem cells capable of expressing a second member of a ligand receptor binding pair. The cells are administered under conditions whereby binding of the ligand to the receptor occurs thereby homing the stem cells to the stromal cells.

Detail Description Paragraph:
[0021] In this embodiment, either the stem cells or the stromal cells are genetically-engineered to provide the capability of expressing the appropriate ligand or receptor. When the methods are used to treat a host afflicted with a bone marrow associated disease, a therapeutically effective amount of stromal cells capable of expressing a first member of a ligand receptor binding pair and a therapeutically effective amount of hematopoietic stem cells capable of expressing a second member of the ligand receptor binding pair are administered to the host.

Detail Description Paragraph:
[0023] The hematopoietic stem cells and bone marrow stromal cells are preferably derived from the species of host being treated or can be derived from a species which does not invoke significant immune responses in the host. The stem cells or stromal cells can also be derived from the host if the cells are functioning properly. The stromal and/or stem cells can be removed from the host and cultured using conventional techniques. Examples of human haematpoietic stem cell lines which can be used in this invention include nonadherent cells derived from human long-term bone marrow cultures. See Greenberg, H. M., et al, Blood, 1981, Vol. 58, pp 724-732, the contents of which are hereby incorporated by reference. Examples of human bone marrow stromal cells include KM101, KM102, KM103, KM104 and KM105. See Fitzgerald et al, Int. J. Radiation Oncology Biol. Phys. Vol 15, pp 1153-59 (1988), the contents of which are hereby incorporated by reference.

Detail Description Paragraph:
[0024] The hematopoietic stem cells or stromal cells can be genetically-engineered using conventional techniques. The DNA encoding the desired ligand or receptor can be inserted into a vector and introduced unto the cells using techniques such as electroporation and/or retroviral infection. Other techniques which can be used to introduce DNA into the cells are calcium phosphate precipitation (Graham and van der Eb, Virology 52:456 (1973) and DEAE-dextran (Cullen et al., Nature 307:241 (1984)).

Detail Description Paragraph:
[0025] The ligand-receptor binding pair are substances having an affinity for each other. At least one member of the ligand-receptor pair is proteinaceous. Examples of ligand-receptor binding pairs include transforming growth factor (TGF) and

transforming growth factor receptor (TGFR) or EGF Receptor; (EGFR) epidermal growth factor (EGF) and EGFR; tumor necrosis factor-.alpha. (TNF-.alpha.) and tumor necrosis factor-receptor (TNFR); interferon and interferon receptor; platelet derived growth factor (PDGF) and PDGF receptor; transferrin and transferrin receptor; avidin and biotin or antibiotin; antibody and antigen pairs; interleukin and interleukin receptor (including types 3, 4 and 5); granulocyte-macrophage colony stimulating factor (GMCSF) and GMCSF receptor; macrophage colony stimulating factor (MCSF) and MCSF receptor; and granulocyte colony stimulating factor (G-CSF) and G-CSF receptor. Further, the ligand-binding pair can be a pair wherein the first member is naturally-occurring and the second member is provided using genetic-engineering techniques. For example, the stromal cells can be genetically-engineered by inserting DNA encoding sugar receptors and this will enhance the homing of the stem cells to the stromal cells based upon the naturally-occurring sugar molecules present in stem cells (Aizawa et al; Exp. Hematol. 16: 811-813 (1988).

Detail Description Paragraph:
[0027] The cells can be administered by subcutaneous or other injection or intraveneously. In methods for treating a host afflicted with a bone marrow associated disease, a therapeutically effective amount of stem cells or stromal cells is that amount sufficient to significantly reduced or eliminate the symptoms or effects of a bone,marrow associated disease. The therapeutically effective amount administered to a host will be determined on an individual basis and will be based, at least in part, on consideration of the individual's size, the severity of symptoms to be treated, and the results sought. Thus, a therapeutic effective amount can be determined by one of ordinary skill in the art of employing such practice in using no more than routine experimentation.

Detail Description Paragraph:
Generation of a Bone Marrow Stromal Cell Line and Purified Hematopoietic Stem Cells

Detail Description Paragraph:
[0029] Human bone marrow stromal cell lines can be established using the technique described by Fitzgerald et al (1988) cited supra and Harigaya et al, PNAS USA 83: 3477-3488 (1985). Human stem cells can be purified from long-term bone marrow cultures using the techniques described in Greenberg, H. M.; et al, Blood, 1981, Vol. 58, pp 724-732, the contents of which are hereby incorporated by reference.

Detail Description Paragraph:
[0030] The vectors, pZipTGF.alpha. and pZipSV(x), were constructed as previously described (Finzi et al., PNAS USA, 84: 3733-37, (1987); William et al, Nature, 310: 476-78 (1984). Murine GBl/6 stromal cells were transfected with pZipTGF-.alpha. using electroporation as described by Pierce et al. (Science, 239: 628-31 (1988)). After twenty-four hours, the medium was replaced and the cells were selected for pZipTGF.alpha. transfectants in 1 mg/ml G418 (Gieneticin GIBCO). Cells which were resistant to G418 were expanded and assayed for TGF-.alpha. production. The cells containing the DNA encoding TGF-.alpha. were labeled GP-TGF-.alpha.. A control cell population was generated by defective retroviral vector infection of the urine GBl/6 cell line using 24-hour culture supernatants from 4.sub.2 cells transfected with the PZip/neo DNA. This control cell population was labeled GBlneo.RTM..

Detail Description Paragraph:
Generation of a Stromal Cell Line that Expresses pro TGF-.alpha. on the Cell Surface

Detail Description Paragraph:
[0033] The murine bone marrow stromal cell line, GBl/6, supports myelopoiesis of enriched progenitors from long-term bone marrow cultures, but does not support adhesion or proliferation of the interleukin-3 (IL-3)-dependent hematopoietic

progenitor cell line, 32D (Anklesaria et al, Proc. Natl. Acad. Sci. USA 84, 7681-7685 1987); Greenberger et al, cited supra). GB1/6 cells were transfected with the retrovirus expression vector pZip-TGF.alpha. containing the entire coding region for human proTGF-.alpha. transcribed under the control of a retroviral LTR (Finzi et al, cited supra (1987)) to obtain a GB1/6 derivative that would express pro-TGF-.alpha.. The same vector lacking the proTGF-.alpha. cDNA insert, pZIP/neo, was introduced into GB1/6 to generate a control cell population. The resulting cells expressing these vectors were designated GP-TGF-.alpha. and GBlneo.RTM., respectively, and were selected for resistance to G418 conferred by expression of the bacterial transposon Tn5 neomycin resistance gene (Cepko et al, Cell 37:1653-62 (1984)) present in the vectors. Both cell mass populations were expanded and analyzed for their ability to express proTGF-.alpha.. Northern blot analysis demonstrated the presence of proTGF-.alpha. mRNA with the expected 4.8 kb size in GP-TGF-.alpha. cells, and no detectable expression of the endogenous TGF-.alpha. gene in GBlneo.RTM. cells (FIG. 2).

Detail Description Paragraph:
Homing of A Genetically-Engineered Hematopoietic Cell Line Expressing the EGF Receptor to Stromal Cells Expressing proTGF.alpha.

Detail Description Paragraph:
[0042] 32D-EGFR cells were cocultivated with confluent monolayers of GP-TGF-.alpha. stromal cells in the absence of any added IL-3 or EGF. Within four to six days, the 32D-EGFR cells began to form foci of flattened adherent cells with approximately 10 cells/focus (FIG. 6). The morphology of these foci was typical of the "cobblestone islands" generated by primary cultures of bone marrow haematpoietic progenitors and stromal cells (Dexter et al, J. Cell. Physiol. 91, 335-344 1977; Williams et al, J. Cell. Physiol. 102, 287-295 1977; Greenberger, cited supra; Anklesaria et al, cited supra). The 32D-EGFR cell islands progressively increased in size (>25 cells/island) and number between days 6 and 40 of cocultivation (FIG. 3A, a bars). Cell adhesion and island formation were not detected when 32D-EGFR cells were cocultivated with GBlneo.RTM. cells, or when 32D cells were cocultivated with GP-TGF-.alpha. cells or GBlneo cells.RTM. (FIG. 3A, bars b, c and d).

Detail Description Paragraph:
[0043] In addition to attachment to the stromal layer, the adherent foci of 32D-EGFR cells were able to continuously release viable hematopoietic cells into the culture medium- for at least forty days in culture (FIG. 3B, .alpha. bars). Cells released into culture medium had the phenotype of normal 32D-EGFR cells (Pierce et al, 1988, cited supra) as determined by their ability to respond to both EGF and IL-3, and to form colonies in semisolid medium (data not shown). In contrast, there were no viable cells (FIG. 3B, bars b and d) or less than 1% of the initial innoculum (FIG. 3B, c bars) produced when hematopoietic-stromal cells were cocultivated in combinations other than 32D-EGFR/GP-TGF-.alpha.. Hematopoietic cells plated along in serum-supplement medium without IL-3 or EGF lost viability within forty-eight to seventy-two hours. Other control flasks containing monolayers of stromal cells alone had fewer than 0.1% of the cells in the culture supernatant.

Detail Description Paragraph:
[0049] To distinguish between these two possibilities, 5-bromo-2' deoxyuridine (BUdR) was added to nine day old and twenty-one day old cocultures and allowed to incorporate into relicating DNA for various lengths of time. To visualize and quantiate cells that had undergone DNA replication during the time of exposure to BUdR, monolayers containing adherent 32D-EGFR cells and supernatants containing non-adherent cells were fixed and stained for indirect immunofluorescence with anti-BUdR andibody and rhodamine-conjugated secondary antibody. The stromal cells were essentially quiescent with fewer than 0.5% of the nuclei becoming labeled under any of the conditions tested (FIG. 8 and Table 2). A significant proporation of adherent 32D-EGFR cells incorporated BUdR into the nucleus (FIG. 8 and Table 2).

In nine day old cocultures, this proportion increased progressively with time of exposure to BUdR, reaching 38% of the adherent cells after a twenty-four hour exposure to BUdR. In twenty-one day old cocultures, as many as 30% of the cells became labeled after only three hours of exposure to BUdR, but this proportion increased slowly with extended labeling times (Table 2). In contrast to the high labeling index observed in adherent cells, only 10% or fewer of the non-adherent cells recovered fromn the cocultures became labeled (Table 2). This number included any cells that detached from the monolayers during collection of the media at the end of the labeling period. Furthermore, removal of the non-adherent cells from the cultures before a short (three hours) labeling of the cell layers with BUdR had essentially no effect on the proportion of adherent 32D-EGFR cells containing labeled nuclei (Table 2). From these results, it was concluded that 32D-EGFR cells replicated their DNA while they were bound to membrane proTGF-.alpha. on the stromal cell monolayer.

Detail Description Paragraph:
RECOVERY OF DONOR ORIGIN STROMAL (GPTGF.alpha.) AND HEMATOPOIETIC (32DEGFR) CELLS FROM TRANSPLANTED MICE

Detail Description Paragraph:
[0050] The presence of donor origin stromal cells were detected by plating fresh bone marrow from control and transplanted mice (9 weeks after transplant with GPTGF.alpha. cells and 1 week after a second transplant of 32D-EGF-R cells) at 5.times.10.sup.5 cells/60 mm dish and selecting with 300 .mu.g/ml of G418. Results are shown in Table 3 as the mean=SD of 3 plates/hind limb from each of the three to five mice per group.

Detail Description Paragraph:
[0052] The data indicates that C57BL/6J mice are stably engrafted in vivo with a clonal stromal cell line producing recombinant TGF.alpha. (GP-TGF.alpha.) to provide an in vivo microenvironment to which 32D-EGFR cells will home. As shown in Table 3, mice prepared for high dose irradiation of both hind limbs and a total body irradiation dose which was sublethal were engrafted in vivo by intravenous injection of the GP-TGF.alpha. cell line. Six months later, the animals received a total body irradiation dose and injection intravenously, of 32D-EGFR cells. Donor origin stromal cells were then measured several week later by explant of adherent cells showing neo.RTM. resistance (a selection marker linked to the TGF.alpha. construct) and hematopoietic cells showing mycophenolic acid resistance (a resistance gene linked to the EGFR construct). The controls included animals irradiated and injected with the stromal cell line only, and other animals irradiated and injected with the TGF.alpha. producing stromal cell line and then with the 32D cell line that did not have the EGFR receptor. The results showed that only in combination of GP-TGF.alpha. cell line engrafted, and then 32D EGFR inoculation, was there evidence for survival of 32D-EGFR cells in vivo. Furthermore, these cells were only detected at sites of stable engraftment of GP-TGF.alpha. stromal cells. Mice not injected with the stromal cell line, but inoculated with 32-EGFR cells, showed no detectable hematopoietic cells at the same time interval. Thus, the data provide in vivo evidence for the engraftment of stromal cell lines followed by homing of hematopoietic stem cell lines using the receptor ligand interaction as the mechanism for homing of hematopoietic to the stromal cells in

Detail Description Table CWU:
3TABLE 3 TOTAL NUMBER (% RESISTANT)* HEMATOPOIETIC CELLS 125.sub.I - EGF STROMAL CELLS Cells/limb bound G418.sup.I Mycophenolic acid.sup.I .times. cpm/10.sup.5 MICE CFU-F/LIMB 10.sup.5 cells Control RHL 2.0 .+-. 1.9 0.8 .+-. 0.45 0.7 .+-. 0.1 - GPTGF.alpha. (3.0 .+-. 2.9%) (2.2 .+-. 1.2%) +32DEGFR LHL 0 1.3 .+-. 0.96 N.T. (0) (3.6 .+-. 2.6%) TRANS- RHL 11.3 .+-. 1.7** 9.2 .+-. 3.4 4.7 .+-. 0.16 PLANTED +GPTGF.alpha. (17.3 .+-. 2.6% (30 .+-. 11%) +32DEGFR LHL 11.0 .+-. 3.0** 15.2 .+-. 8.0 N.T. (16.9 .+-. 4.6%) (51 .+-. 26%) *% resistant cells: number of cells

recovered in presence of drug/number of cells in absence of drug .times. 100 **p < 0.05 compared to control mice.

CLAIMS:

1. A method for homing hematopoietic stem cells to bone marrow stromal cells in a host, comprising: administering to the host genetically-engineered hematopoietic stem cells capable of expressing a first member of a ligand-receptor binding pair under conditions whereby binding of the first member of the ligand-receptor binding pair to a second member of the ligand-receptor binding pair, present on stromal cells, occurs thereby homing the stem cells to the stromal cells.

2. A method for homing hematopoietic stem cells to bone marrow stromal cells in a host, comprising: administering to the host stromal cells capable of expressing a first member of a ligand-receptor binding pair; and subsequently administering to the host hematopoietic stem cells capable of expressing a second member of a ligand-receptor binding pair under conditions whereby binding of the ligand to the receptor occurs thereby homing the stem cells to the stromal cells, wherein either the stromal cells or the hematopoietic stem cells are genetically-engineered to provide the capability of expressing the appropriate ligand or receptor.

5. A method according to claim 2 wherein the bone marrow stromal cells are selected from the group consisting of GBL/6, KM101, KM102, KM103, KM104 and K105.

6. A method according to claim 2 wherein the stromal cells are capable of expressing a ligand.

10. A method according to claim 2 wherein either the genetically-engineered stromal cells or hematopoietic stem cells are produced by transfecting the cells with a retroviral vector containing RNA which is reverse transcribed to DNA encoding a member of a ligand-receptor binding pair.

11. A method for transplanting bone marrow in a host, comprising: administering to the host stromal cells capable of expressing a first member of a ligand-receptor binding pair; and administering to the host hematopoietic stem cells capable of expressing a second member of a ligand-receptor binding pair under conditions whereby binding of the ligand to the receptor occurs thereby homing the stem cells to the stromal cells, wherein either the stromal cells or hematopoietic stem cells are genetically-engineered to provide the capability of expressing the appropriate ligand or receptor.

14. A method according to claim 11 wherein the bone marrow stromal cells are selected from the group consisting of GBL/6, KM101, KM102, KM103, KM104 and KM105.

15. A method according to claim 11 wherein the stromal cells are capable of expressing a ligand.

19. A method according to claim 11 wherein the genetically-engineered stromal cells or the hematopoietic stem cells are produced by transfecting the cells with a retroviral vector containing RNA which is reverse transcribed to DNA encoding a member of a ligand-receptor binding pair.

20. A method of treating a host afflicted with a disease associated with a disorder of the bone marrow, comprising: administering to the host a therapeutically effective amount of stromal cells capable of expressing a first member of a ligand-receptor binding pair; and administering to the host a therapeutically effective amount of hematopoietic stem cells capable of expressing a second member of a ligand-receptor binding pair under conditions whereby binding of the ligand to the receptor occurs, thereby homing the stem cells to the stromal cells, wherein either the stromal cells or hematopoietic stem cells are genetically-engineered to provide

the capability of expressing the appropriate ligand or receptor.

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L10: Entry 160 of 237 File: USPT Sep 19, 2000

DOCUMENT-IDENTIFIER: US 6121270 A
TITLE: Epoxide-containing compounds

Brief Summary Text (16):
The invention includes a method for modulating an immune response or a cellular response to external or in situ primary stimuli comprising administering an effective amount of an inventive compound. Particularly, on a cellular or biochemical level, the inventive compounds have been found to inhibit a specific phospholipid-based pathway that amplifies a signal within a cell. This pathway tends to be activated in response to noxious or inflammatory stimuli. The inventive compounds also decrease proliferation of tumor cells in response to an activated oncogene; stimulate hematopoiesis in the presence of agents which inhibit hematopoiesis, such as chemotherapeutic agents; suppress the activation of T-cells in the presence of antigen and the secretion of antibodies by B-cells in the presence of antigen; suppress the activation of macrophage or endothelial cells by endotoxins, tumor necrosis factor (TNF), onterleukin-1 (IL-1) or granulocyte macrophage colony stimulating factor (GM-CSF); enhance the resistance of mesenchymal cells to TNF; inhibit the proliferation of smooth muscle cells, endothelial cells, fibroblasts and other cell types in response to growth factors, such as platelet derived growth factor (PDGF), PDGF-AA, PDGF-BB, fibroblast growth factor (FGF), epidermal growth factor (EGF), etc.; inhibit the activation of T-cells and viral replication in response to human immunodeficiency virus; inhibit the proliferation of kidney mesangial cells in response to IL-1; prevent suppression of Steel factor (also called stem cell factor, mast cell growth factor and kit ligand), granulocyte colony stimulating factor (G-CSF), oncostatin M or interleukin-6 (IL-6) in bone marrow stromal cells in response to TNF; suppress expression of adhesion molecules in endothelial cells and suppress adhesion of inflammation cells to endothelial cells; suppress proliferation of kidney mesangial cells in response to IL-1, mip-1.alpha., PDGF or FGF; prevent toxicity in kidney glomerular or tubular cells in response to cyclosporin A or amphotericin B; prevent cytotoxic effects in gastrointestinal or pulmonary epithelial cells in response to a cytotoxic drug or radiation; enhance the antitumor effects in tumor cells in response to a nonalkylating antitumor agent; suppress the production of metalloproteases in synovial cells, other fibroblasts and a glomerular epithelial cell in response to inflammatory stimuli, such as TNF, IL-1 and the like; inhibit production of osteoclast-activating factor (OAP) by osteoclasts in response to IL-1; inhibit degranulation of mast cells and basophils in response to IgE; modulate signal transduction of the neurotransmitters epinephrine and acetylcholine in neural pathways utilizing these transmitters, block activation of platelet activating factor in inflammation cells, block release of TNF and IL-1 in various cell types in response to inflammatory stimuli, block activation and proliferation of lymphocytes and other cell types to IL-1 and interleukin-2 (IL-2), and the like, including the clinical manifestations of these cellular and biochemical events.

Drawing Description Text (5):
FIG. 4 shows a comparison of 1605, 1808 and 1906 on PDGF-induced (platelet derived growth factor) proliferation of human stromal cells. Human stromal cells were starved in serum-free media for 24 hours and then stimulated with 50 ng/ml of PDGF-BB. The drugs were added at various indicated concentrations one hour prior to PDGF stimulation. Tritiated thymidine was added for 24 hrs at the time of PDGF

stimulation to measure cellular proliferation. Background counts were approximately 5% of control levels. All three drugs inhibited PDGF-induced stimulation in a dose response fashion.

Detailed Description Text (265):
This example illustrates the effects of inventive compounds nos. 1114, 1413, 1560, 1565, 1594, 2518, 2548R, 2548S and 3503 as effective inhibitors of normal human bone marrow stromal cells (MSC) proliferation in response to Platelet Derived Growth Factor BB (PDGF B) and IL-1.alpha. (50 and 10 ng/ml, respectively). Maximum proliferation occurs when both PDGF B and IL-1.alpha. are present, hence a combination of both were used in the following assay.

Detailed Description Text (277):
This example illustrates a comparison of inventive compounds nos. 1605, 1808 and 1906 on PDGF-induced (platelet derived growth factor) proliferation of human stromal cells. Human stromal cells were starved in serum-free media for 24 hours and then stimulated with 50 ng/ml of PDGF-BB. The drugs were added at various indicated concentrations one hour prior to PDGF stimulation. Tritiated thymidine was added for 24 hours at the time of PDGF stimulation to measure cellular proliferation. Background counts were approximately 5% of control levels. As shown in FIG. 4, all three drugs inhibited PDGF-induced stimulation in a dose response fashion.

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L10: Entry 164 of 237 File: USPT Jan 18, 2000

DOCUMENT-IDENTIFIER: US 6015554 A
TITLE: Method of reconstituting human lymphoid and dendritic cells

Detailed Description Text (20):
Once the RA.sup.+ and/or 10.sup.+ cells have been isolated, they may be propagated by growing in conditioned medium from stromal cells, such as stromal cells that can be obtained from bone marrow, fetal thymus or fetal liver, and are shown to provide for the secretion of growth factors associated with progenitor cell maintenance, or co-culturing with such stromal cells, where the stromal cells may be autologous, allogeneic or xenogeneic. Before using in the co-culture, the mixed stromal cell preparations may be freed of hematopoietic cells employing irradiation, cytotoxic drugs or appropriate monoclonal antibodies for removal of the undesired cells, e.g., with antibody-toxin conjugates, antibody and complement, etc. Alternatively, cloned stromal cell lines may be used where the stromal lines may be allogeneic or xenogeneic.

Detailed Description Text (25):
Further demonstration of the differentiative capacity of the various cell populations might be accomplished by the detection of myeloid and NK cell production in the stromal cell assays described in the Examples that follow.

Detailed Description Text (68):
AC6.21 stromal cell monolayers were established one week prior to the experiment by plating 1.times.10.sup.4 AC6.21 cells per well of a 96-well flat bottom plate in 100 .mu.l of medium consisting of 50% IMDM (JRH Biosciences), 50% RPMI with 10% FCS (Hyclone), 4.times.10.sup.-5 M (2-mercaptoethanol, 10 mM HEPES, penicillin (100 U/ml), streptomycin (100 .mu.g,/ml) (P/S) and 4 mM glutamine (JRH Biosciences). Sorted cells were distributed at 100 cells per well on the pre-established AC6.21 cell monolayer in medium containing IL-3 (10 ng/ml), IL-6 (10 ng/ml) and leukemia inhibitory factor (LIF) (50 ng/ml) (Sandoz Pharma). Half of the cytokine-containing medium was replaced weekly. At the end of the three week long culture, cells were harvested by pipetting, counted and transferred to subsequent assays.

Detailed Description Text (74):
Co-cultivation of ABM subsets on the murine bone marrow stromal cell line AC6.21 was done in the presence of IL-3, IL-6 and LIF. The addition of cytokines to the co-culture was necessary to observe optimum growth of adult cells. After 3 weeks, both RA.sup.- and RA.sup.+ cells expanded well (respectively 100- to 500- and 70- to 200-fold the input cell number in 3 experiments), and both subsets produced visibly differentiated cells; however, cultures of RA.sup.- cells displayed more clusters of small blasts resembling cobblestone areas.

Detailed Description Text (84):
CD34.sup.+ Lin.sup.- CD10.sup.+ cells were also evaluated in stroma-supported bone marrow cultures. Sorted cells were co-cultivated on murine AC6.21 stromal cells in the presence of IL-3, IL-6 and leukemia inhibitory factor (LIF), a cytokine combination known to support the growth of adult hematopoietic cells as described by Murray et al. (1994). After 3 weeks, CD34.sup.+ Lin.sup.- CD10.sup.- cells expanded considerably (174 to 500 fold total cellular expansion) and 3-18% CD34.sup.+ cells were still present in the culture, indicating some retention of

primitive hematopoietic cells. Morphologically heterogeneous populations of myeloid cells were observed. The great majority of cultured cells (86-97%) expressed the myeloid antigen CD33, with no detectable CD19.sup.+ lymphoid cells.

Detailed Description Text (90):
The capacity to differentiate into B-cells was analyzed after co-culture on AC6.21 murine bone marrow stromal cells known to support human B-cell differentiation from fetal and adult bone marrow CD34.sup.+ Lin.sup.- cells. Baum et al. (1992); and DiGiusto et al. (1994). As described above, RA.sup.+ and RA.sup.- cells grew on AC6.21 cells in the presence of IL-3, IL-6 and LIF. Cultures derived from RA.sup.+ and RA.sup.- cells were almost exclusively composed of CD33.sup.+ myeloid cells (Table 4).

Detailed Description Text (108):
It has recently been demonstrated that NK cells can be differentiated from CD34.sup.+ bone marrow cells with IL-2 and stroma. Miller et al. (1992) Blood 80:2182-2187; and Lotzova et al. (1993) J. Immunol. 150:5263-5269. The ability of the murine stromal cell line AC6.21 to support NK cell differentiation was examined. Cells sorted into CD34.sup.+ Lin.sup.- subsets, as described in Example 1, were assayed for NK cell differentiation as follows.

Detailed Description Text (110):
For NK cell assays, sorted cells were plated onto preformed AC6.21 monolayers in IMDM with 10% FCS, 40 .mu.g/ml transferrin (Boehringer Mannheim), 5 .mu.g/ml insulin (Sigma) supplemented with 50 ng/ml recombinant human IL-2 (Sandoz Pharma, Basel Switzerland). After 1 week, half of the medium was replaced by medium containing rhIL-2 (50 ng/ml). Subsequently, medium containing IL-2 was changed twice a week for at least two weeks to supplement for the disappearance of stromal cells. Cells were harvested by pipetting, counted and immunostained with PE-conjugated anti-CD56 and FITC conjugated anti-CD3 MABs or their appropriate negative controls (Becton Dickinson). Cells were analyzed on a FACScan fluorescent cell analyzer (Becton Dickinson).

Detailed Description Text (111):
Under these conditions, within 2 too 3 weeks, RA.sup.+ cells developed into lymphoblasts expressing CD56 but not CD3 (FIGS. 9 and 10). Positivity for CD56 and lack of detectable surface CD3 expression is typical of NK cells. Lanier et al. (1992) Immunology Today 13:392-395. RA.sup.+ cultures generally destroyed their supportive stromal layer within the first two weeks as described by Miller et al. (1992). Furthermore, the 10.sup.+ subset, which has almost no myeloid potential but has T and B-cell progenitor activity described Example 2, can generate NK cells (FIG. 11). The CD56+CD3.sup.- NK cells produced in the culture also destroyed the stroma.

Detailed Description Text (116):
Differentiation of ABM CD34.sup.+ Lin.sup.- CD10.sup.+ cells into CD34.sup.- CD56.sup.+ CD3.sup.- NK cells was obtained within 1 to 2 weeks of culture on the murine bone marrow stromal cell line AC6.21 in the presence of IL-2 in all experiments performed (n=6). NK differentiation potential of CD34.sup.+ Lin.sup.- CD10.sup.+ cells was confirmed with highly purified cells obtained after two consecutive rounds of flow cytometric sorting.

Other Reference Publication (67):
Paul et al., "Stromal cell-associated hematopoiesis: Immortalization and characterization of a primate bone marrow-derived stromal cell line" Blood (1991) 77:1723-1733.

Other Reference Publication (73):
Whitlock et al., "Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule" Cell (1987) 48:1009-

1021.

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L10: Entry 175 of 237 File: USPT Nov 3, 1998

DOCUMENT-IDENTIFIER: US 5830682 A
TITLE: Preparation of immortalized cells

Brief Summary Text (26):
In another aspect, the invention provides immortalized prostatic lineage cell lines from a growth suppressor deficient mammal. Preferably the cell lines are from a P53 deficient mouse. The cell lines include epithelial lineage cell lines characterized by expression of cytokeratins. The cell lines also include stromal lineage cell lines characterized by expression of smooth-muscle actin or prolyl-4-hydroxylase.

Detailed Description Text (129):
Isolation of Immortalized Prostatic Epithelial and Stromal Lineage Cell Lines

Detailed Description Text (130):
Prostate tissue contains epithelial and stromal cells. There are three subtypes of epithelial cells: (1) secretory luminal cells, which line the prostate ducts, express cytokeratins 8 and 18, and in humans, produce PSA and other prostatic products; (2) basal cells, which rest on the basement membrane, express cytokeratins 5 and 14, (and are considered by some to be the "stem" cells) (Peehl et al., Cell Tissue Res. 277, 11-18 (1994); and (3) endocrine/paracrine cells, which reside among the secretory cells in all parts of the prostate. The origin and function of these cells are currently unknown. They can be identified by the expression of chromogranin A (Bonkhoff & Remberger, The Prostate 28, 98-106 (1996)). Stromal cells are subdivided into fibroblasts (detected by prolyl 4-hydroxylase) and smooth muscle cells (detected by smooth-muscle actin) (Kassen et al., The Prostate 28, 89-97 (1996).

Detailed Description Text (131):
Prostatic epithelial and stromal cells can be obtained from mammals such as P53-deficient mice by established procedures. See Orlowski et al., J. Androl. 3:232-240; Montpetit, Prostate 15, 315-325; Turner, In Vitro Cell Dev. Biol. 26, 722-730; Kassen, Prostate 28, 89-97. Prostatic tissue is minced into small fragments, and the fragments digested with 1% collagenase, 1% trypsin and 0.1% hyaluronidase at 37.degree. C. for 3-4 hr. The cells are collected by centrifugation and added to the top of a preformed Percoll density step gradient. Cells from different fractions of the gradient are plated on fibronectin-coated plates in RPMI 1640 medium containing 10% fetal calf serum and characterized by their morphology. Cells with an epithelial morphology are grown in DMEM/F12 medium containing 10 ng/ml cholera toxin, 10 ng/ml epidermal growth factor, 4 .mu.g/ml insulin, 10 .mu.g/ml transferrin, 1 .mu.g/ml hydrocortisone, 1 .mu.g/ml dihydrotestosterone, 100 .mu.g/ml bovine pituitary extract, 100 .mu.M phosphoethanolamine, 30 nM selenous acid, and 2.3 .mu.M .alpha.-tocopherol. Turner et al., supra; Peehl et al., In Vitro Cell Dev. Biol. 24, 530-536.) Cells without an epithelial morphology (stromal cells) are grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (Kassen, supra). Cells surviving about 80 passages are immortalized. Cells can be verified for retention of differentiation markers at intervals through the passaging. Immortalized cell lines can be promulgated from isolates of the surviving cells. The identity of the cell types isolated is verified by immunostaining with antibodies to cytokeratins (epithelial cells), smooth-muscle actin (smooth muscle cells), and prolyl-4-hydroxylase (fibroblasts). The three

types of epithelial cells can be distinguished by the differentiation markers noted above. Peehl et al., supra; Bonkhoff & Remberger, supra; Kassen et al., supra.

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L10: Entry 184 of 237 File: USPT Jun 9, 1998

DOCUMENT-IDENTIFIER: US 5763197 A
TITLE: Human hematopoietic stem cell

Brief Summary Text (31):
The subject compositions are found to provide for production of myeloid cells and lymphoid cells in appropriate cultures, cultures providing hydrocortisone for production of myeloid cells (associated with Dexter-type cultures) and B lymphocytes in cultures lacking hydrocortisone, (associated with Whitlock-Witte type cultures). In each of the cultures, mouse or human stromal cells are provided, which may come from various strains, AC3 or AC6, stromal cells derived from mouse or human fetal bone marrow by selection for the ability to maintain human stem cells, and the like. The medium employed for the culturing of the cells is conveniently a defined enriched medium, such as IMDM (Iscove fs Modified Dulbecco's Medium), a 50:50 mixture of IMDM and RPMI, and will generally be composed of salts, amino acids, vitamins, 5.times.10.sup.-5 M 2-ME, streptomycin/penicillin and 10% fetal calf serum, and may be changed from time to time, generally at least about once to twice per week. Particularly, by transferring cells from one culture with hydrocortisone, to the other culture without hydrocortisone, and demonstrating the production of members of the different lineages in the different cultures, the presence of the stem cell and its maintenance is supported. In this manner, one may identify the production of both myeloid cells and B-cells.

Brief Summary Text (36):
Once stem cells have been isolated, they may be propagated by growing in conditioned medium from stromal cells, such as stromal cells that can be obtained from bone marrow, fetal thymus or fetal liver, and are shown to provide for the secretion of growth factors associated with stem cell maintenance, coculturing with such stromal cells, or in medium comprising maintenance factors supporting the proliferation of stem cells, where the stromal cells may be allogeneic or xenogeneic. Before using in the coculture, the mixed stromal cell preparations may be freed of hematopoietic cells employing appropriate monoclonal antibodies for removal of the undesired cells, e.g., with antibody-toxin conjugates, antibody and complement, etc. Alternatively, cloned stromal cell lines may be used where the stromal lines may be allogeneic or xenogeneic.

Brief Summary Text (37):
The subject cell compositions may find use in a variety of ways. Since the cells are naive, they can be used to reconstitute fully an irradiated host and/or a host subject to chemotherapy; or as a source of cells for specific lineages, by providing for their maturation, proliferation and differentiation into one or more selected lineages by employing a variety of factors, such as erythropoietin, colony stimulating factors, e.g., GM-CSF, G-CSF, or M-CSF, interleukins, e.g., IL-1, -2, -3, -4, -5, -6, -7, -8, etc., Leukemia Inhibitory Factory (LIF), Steel Factor (St1), or the like, or stromal cells associated with the stem cells becoming committed to a particular lineage, or with their proliferation, maturation and differentiation. The stem cells may also be used in the isolation and evaluation of factors associated with the differentiation and maturation of hematopoietic cells. Thus, the stem cells may be used in assays to determine the activity of media, such as conditioned media, evaluate fluids for cell growth activity, involvement with dedication of particular lineages, or the like.

Brief Summary Text (41):
The cells may be frozen at liquid nitrogen temperatures and stored for long periods of time, being thawed and capable of being reused. The cells will usually be stored in 10% DMSO, 70% autologous plasma (irradiated with 2500 rad), 20% Tc199 (Tissue culture medium). Cells are frozen in a programmable cell freezer to -180.degree. C. in liquid nitrogen. Once thawed, the cells may be expanded by use of growth factors or stromal cells associated with stem cell proliferation and differentiation.

Detailed Description Text (9):
Various murine stromal cell lines were employed, three of which are described in Whitlock et al., Cell (1987) 48:1009-1021. Confluent stromal cell layers were maintained for up to 3-4 weeks without passage by changing of the tissue culture medium every 5-7 days. To passage, the stromal cell layers were washed 3 times with serum-free medium, then overlaid with 2.5 ml (T-25 flask) of 0.5 mg/ml collagenase-dispase (Boehringer-Mannheim, Indianapolis, Ind.) in serum-free medium. The cultures were allowed to incubate 15-30 minutes at 37.degree. C.; then the cells in the enzyme-containing medium were collected and RPMI-1640 medium with serum added. The stromal cells were suspended by pipetting with a Pasteur pipet, then cultured directly at 1/5th to 1/50th the original cell concentration. In general, confluent stromal layers subcultured at 1:10 reached confluency again after 5-7 days. Subclones were obtained by limiting dilution culture from 30 to 0.3 cells per well. Human stromal cell lines were treated similarly.

Detailed Description Text (13):
Bulk cultures are obtained by placing the human cells on the previously established confluent layer of mouse or human stromal cell lines. From 3.times.10.sup.4 to 2.times.10.sup.5 cells per ml are placed on the stromal cells in either T-25 flasks or 6 well plates, by addition of 3 ml to each well of a 6 well plate or 5 ml to T-25 flask. A 50:50 mixture of RPMI-1640 and IMDM containing 50 .mu.g/ml penicillin/50 .mu.g/ml streptomycin, 1 mM sodium pyruvate, 2 mM glutamine, 5.times.10.sup.-5 2-mercaptoethanol and 10% fetal calf serum is employed. For Dexter-type conditions, IMDM containing 50 .mu.g/ml penicillin/50 .mu.g/ml streptomycin, 1 mM sodium pyruvate, 2 mM glutamine, 10% fetal calf serum, 20% horse serum and 10.sup.-6 M hydrocortisone sodium succinate is employed. Bone marrow cells grown in the Dexter-type medium give rise only to myeloid differentiation. Cultures were established with whole cell populations or cells fractionated by their expression of cell surface antigens (CD34, HLA-DR, Thy-1, Lineage markers).

Detailed Description Text (14):
Limiting dilution cultures were prepared using 96 well plates containing the mouse stromal cells as confluent layers. The human cells were titered into the plates at progressively lower concentrations with at least 24 wells plated at each cell concentration. The plates were then examined to determine the percentage of positive wells at each cell number. The data is then plotted graphically.

Detailed Description Text (16):
Cocultures established with the mouse bone marrow stromal cell lines, AC3 or AC6, have served successfully as feeder layers for human cultures and have inhibited fibroblast overgrowth at low cell densities.

Detailed Description Text (18):
(2) Cultures show small to medium sized human bone marrow cells attached to the mouse stromal cells and proliferation occurs over the first one to three weeks of culture; thereafter they remain fairly stable.

Detailed Description Text (19):
(3) Cells form loose aggregates consisting of non-adherent and adherent cells overlying stromal cells which in turn overlie small to intermediate sized cells (pseudo-emperipolesis). Overall, the appearance of the cultures is similar to mouse

long-term cultures.

Detailed Description Text (24):
In addition, a single cell assay has been developed in which single FACS sorted progenitor cells are placed into individual wells of a 96 well plate containing a mouse or human bone marrow stromal cell feeder layer. Results shown on Table 7 indicate that 1 in 40 CD34+ LIN.sup.- (Lin=CD3, 10, 19, 20, 15, 33) or 1 in 80 CD34+ Lin+ cells respond by colony formation (0.5-1% of bone marrow). Analysis of the colonies show that 40% and 25% of the colonies are multipotent as determined by FACS and methylcellulose analysis (B lymphoid, myeloid, erythroid) in the CD34+ Lin- and CD34+ Lin+ populations respectively. Further, CD34+ Thy+ cells (0.1-0.5% of bone marrow) show colony growth in 1 in 20 wells. The majority of the colonies are multipotent (>70%). The CD34+Thy+ cells are the most efficient population in terms of growth after transfer to new stromal layers. In contrast, the CD34+ Thy- cells, which represent >90% of the CD34+ cells, respond poorly in the co-culture assay; 1 in 400 cells form colonies, none of which are multipotent. The Thy+ population can also be subdivided according to expression of mature lineage markers. The Thy+Lin- cell subset (0.1-0.5% of bone marrow) responds well in the single cell assay (1/20) while the Thy+ Lin+ subsets responds poorly (0/800). FACS and methylcellulose assays show that >70% of the colonies derived from Thy+Lin- cells are multipotent. The above data indicates that "stem cells" are present in the subset of cells which express CD34 and Thy-1 but lack expression of lineage markers. Cells with this phenotype represent fewer than 1 in 1000 whole bone marrow cells.

Detailed Description Paragraph Table (7):
TABLE 7 SINGLE CELL PER WELL EXPERIMENTS

FREQUENCY OF BONE MARROW	FREQUENCY OF GROWTH	MULTIPOTENT SUBPOPULATION	POSITIVE WELLS	COLONIES
CD34+Lin-	1/40	40	CD34+Thy+ 1/20	75
CD34+Thy-	1/400	0	Thy+Lin+ 0/854	0
Thy+Lin-	1/21	76		

CD34+Lin+ 1/80* 25%**

Single cells with the described phenotype were deposited wells of a 96 well plate which contained a bone marrow stromal cell feeder layer. *Numbers represent the frequency of cells which gave rise to colonies of >100 cells. The numbers represent the average of at least three separate experiments constituting at least 10 96 well plates. **Numbers represent the percentage of colonies arising from single cells which contained cells of the Blymphoid (CD10+, CD19+), Myeloid (CD15+, CD33+, GMCFU) and erythroid (BFUe) lineages simultaneously.

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L10: Entry 193 of 237 File: USPT Sep 9, 1997

DOCUMENT-IDENTIFIER: US 5665557 A
** See image for Certificate of Correction **
TITLE: Method of purifying a population of cells enriched for hematopoietic stem cells populations of cells obtained thereby and methods of use thereof

Drawing Description Text (36):
The cells obtained as described above and in the Examples may be used immediately or frozen at liquid nitrogen temperatures and stored for long periods of time, being thawed and capable of being reused. The cells will usually be stored in 10% DMSO, 50% FCS, 40% RPMI 1640 medium. Once thawed, the cells may be expanded by use of growth factors or stromal cells associated with proliferation and differentiation.

Drawing Description Text (45):
Once the CDw109.sup.+ cells have been isolated, they may be propagated on stromal cells, such as stromal cells that can be obtained from bone marrow, fetal thymus or fetal liver, and are shown to provide for the secretion of growth factors associated with progenitor cell maintenance where the stromal cells may be allogeneic or xenogeneic. Before using in the co-culture, the mixed stromal cell preparations may be freed of hematopoietic cells employing appropriate monoclonal antibodies for removal of the undesired cells, e.g., with antibody-toxin conjugates, antibody and complement, etc. Alternatively, cloned stromal cell lines may be used where the stromal lines may be allogeneic or xenogeneic.

Drawing Description Text (46):
The invention also encompasses methods of use of the CDw109.sup.+ cell populations. The subject cell compositions may find use in any method known in the art. Since the cells are naive, they can be used to fully reconstitute an immunocompromised host such as an irradiated host or a host subject to chemotherapy; or as a source of cells for specific lineages, by providing for their maturation, proliferation and differentiation into one or more selected lineages by employing a variety of factors, including, but not limited to, erythropoietin, colony stimulating factors, e.g., GM-CSF, G-CSF, or M-CSF, interleukins, e.g., IL-1, -2, -3, -4, -5, -6, -7, -8, etc., or the like, or stromal cells associated with the stem cells becoming committed to a particular lineage, or with their proliferation, maturation and differentiation. The CDw109.sup.+ cells may also be used in the isolation and evaluation of factors associated with the differentiation and maturation of hematopoietic cells. Thus, the CDw109.sup.+ cells may be used in assays to determine the activity of media, such as conditioned media, evaluate fluids for cell growth activity, involvement with dedication of particular lineages, or the like.

Detailed Description Text (43):
AC6.21 mouse BM stromal cells were plated in Whitlock-Witte media in wells of polystyrene flat-bottom 96 well plates (Corning, Corning, N.Y.), and allowed to form a confluent adherent layer. After 1-2 weeks, sorted cells were plated on top of the confluent AC6.21 cells at limiting dilution (range usually 1000 to 30 cells/well, at least 24 wells per concentration) in long term culture medium (1:1 IMDM/RPMI, JRH BioSciences, Woodland, Calif.) containing 10% FCS (Hyclone Labs, Logan, Utah), 50 U/ml penicillin, 50 .mu.g/ml streptomycin, 2 mM glutamine, 1 mM

sodium pyruvate (JRH BioSciences) and 10.sup.-6 M 2-mercaptoethanol (Sigma, St. Louis, Mo.), in the absence of growth factors, or 100 to 1 cell/well in the presence of purified human recombinant interleukin-6 (IL-6, 10 ng/ml) and leukemia inhibitory factor (LIF, 50 ng/ml), provided by Sandoz Pharma Ltd. (Basel, Switzerland). Cultures were fed weekly with medium containing the same growth factors. In the presence of growth factors, cobblestone areas of more than 50 cells were usually counted from 2-5 weeks. At 5 weeks, cells were harvested and stained with CD33-PE and CD19-FITC or CD34 (HPCA2)-PE (Becton Dickinson) or relevant isotype controls, and analyzed on a FACScan. The frequency of CAFC was calculated by the cell number at which 37% of the wells show no growth of cobblestone areas, using regression, with 95% statistical precision according to the method described by Lefkovits and Waldmann (1984) Immunol. Today 5:265.

Detailed Description Text (45):
These results indicate that cells forming cobblestone areas in 5 week coculture with a murine stromal cell line AC6.21 were exclusively contained within the CDw109.sup.+ subset of CD34.sup.+ cells from FBM. Phenotypic analysis demonstrated a high level of CD34.sup.+ cell maintenance, B cell and myeloid cell co-production after 5 weeks in culture indicating the presence of HSC activity in this CDw109.sup.+ subset. CAFC frequency was enriched in the CDw109.sup.+ subset of CD34.sup.+ cells from ABM compared to the CDw109.sup.- subset. The difference is not as striking as that seen in FBM, which may be due to sort purity.

Other Reference Publication (8):
Whitlock et al., "Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule" Cell (1987) 48:1009-1021.

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L1: Entry 18 of 20 File: USPT Feb 10, 1998

US-PAT-NO: 5716616
DOCUMENT-IDENTIFIER: US 5716616 A

TITLE: Isolated stromal cells for treating diseases, disorders or conditions characterized by bone defects

DATE-ISSUED: February 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Prockop; Darwin J.	Philadelphia	PA		
Pereira; Ruth F.	Lansdowne	PA		
Leeper; Dennis B.	Wynnewood	PA		
O'Hara; Michael D.	Wyncote	PA		

US-CL-CURRENT: 424/93.7; 424/93.71, 424/93.72, 424/93.73

CLAIMS:

We claim:

1. A method of treating a patient who is suffering from a disease, disorder or condition characterized by a bone defect comprising the steps of:

a) obtaining a bone marrow sample from a donor who is not suffering from a disease, disorder or condition characterized by a bone defect;

b) isolating adherent cells from said sample; and,

c) administering by intravenous infusion to said patient an amount of said isolated adherent cells effective to treat said disease, disorder or condition, wherein said patient undergoes bone marrow ablation prior to administration of said isolated adherent cells.

2. The method of claim 1 wherein said adherent cells are administered by intravenous infusion to said patient together with non-adherent cells from a bone marrow sample from a donor who is not suffering from a disease, disorder or condition characterized by a bone defect.

3. The method of claim 1 wherein said disease, disorder or condition is osteogenesis imperfecta.

4. The method of claim 1 wherein said bone defect is due to a collagen gene mutation.

5. The method of claim 1 wherein said donor is syngeneic with said patient.

6. The method of claim 1 wherein said isolated adherent cells are cultured to expand the number of said cells and said expanded culture is administered to said individual.

7. The method of claim 6 wherein said disease, disorder or condition is osteogenesis imperfecta.

8. The method of claim 6 wherein said bone defect is due to a collagen gene mutation.

9. The method of claim 6 wherein said donor is syngeneic with said patient.

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L1: Entry 14 of 20 File: USPT Nov 25, 2003

US-PAT-NO: 6653134
DOCUMENT-IDENTIFIER: US 6653134 B2
**** See image for Certificate of Correction ****

TITLE: Isolated stromal cells for use in the treatment of diseases of the central nervous system

DATE-ISSUED: November 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Prockop; Darwin J.	Philadelphia	PA		
Stokes; David G.	Willow Grove	PA		
Azizi; S. Ausim	Philadelphia	PA		

US-CL-CURRENT: 435/377; 435/366, 435/368, 435/372, 435/373

CLAIMS:

What is claimed is:

1. A method of directing the differentiation neural of an isolated stromal cell into a neural cell, comprising culturing said isolated stromal cell in the presence of a substantially homogeneous population of differentiated neural cells whereby said isolated stromal cell differentiates and acquires the phenotypic characteristics of said differentiated neural cells.

2. The method of claim 1, wherein said differentiated cells are astrocytes.

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